DECLARATION OF SUSAN J. KNOX UNDER 37 C.F.R. § 1.132	Application Number	10/576,568
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	First Named Inventor	Susan J. Knox
	Examiner	Frank I. Choi
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I, Susan J. Knox, M.D., Ph.D., do hereby declare as follows:

1. I am listed as an inventor of the above-referenced patent application. I am currently an Associate Professor at Stanford University.

2. I have been asked to provide evidence of the effect of combining a salt of an inorganic selenium-containing compound and radiation therapy to treat neoplastic disease, to enhance sensitivity of a tumor to radiation, and to treat prostate cancer.

3. Well established mouse models for prostate cancer were used to study the radiosensitizing effect of sodium selenite (SSE) in prostate cancer cells. A murine model was also used to study the radiosensitizing effect of SSE on normal radiosensitive intestinal crypt cells. Immunodeficient (SCID) mice with hormone-independent LAPC-4 (HI-LAPC-4) and PC-3 xenograft tumors (~200 mm^3) were divided in 4 groups: control (untreated), XRT (local irradiation), SSE (2 mg/kg, IP, TIW), and XRT+SSE. The XRT was given at the beginning of the regimen as a single dose of 5 Gy for HI-LAPC-4 tumors, and a single dose of 7 Gy followed by a fractional dose of 3 Gy/day x 5 day for PC-3 tumors. The tumor volume was measured 3 times/week. The radiosensitizing effect

of SSE on normal intestinal epithelial cell was assessed using a crypt cell microcolony assay.

4. SSE alone significantly inhibited the tumor growth in LAPC-4, but not PC-3 tumors. SSE significantly enhanced the XRT-induced tumor growth inhibition in both LAPC-4 and PC-3 tumors. (See Fig. 1). Fig. 1 shows that in untreated control mice, the volume of HI-LAPC-4 and PC-3 prostate cancer xenograft tumors increased steadily over a one month period, with a tumor quadrupling time of approximately 2 weeks. XRT alone significantly inhibited the tumor growth rate. SSE alone significantly inhibited growth in HI-LAPC-4 tumors, but not in PC-3 tumors. The combined treatment of XRT and SSE further enhanced the inhibitory effect of XRT.

5. SSE did not affect the intestinal crypt cell survival either alone or in combination with XRT. (See Figs. 2 and 3). Fig. 2 shows that SSE at the dose of 2, 3.5, and 6.125 mg/kg (Intaperitoneal) for 2 and 4 weeks did not affect the number of crypts/cross section of duodenum, jejunum, ileum, and colon as compared with untreated control animals (Fig 2, Panels A and B). In addition, SSE had no detectable effect on the parameters of the intestinal epithelium growth (crypt depth and villus height) (Fig 2, Panels A and B). SSE did not radiosensitize intestinal epithelium to irradiation. In mice treated with 10-15 Gy XRT, a linear decrease of crypt cells was observed as a function of XRT dose in all sections of the intestine (duodenum, jejunum, ileum, colon, and rectum). The slope of the decrease (indicative of radiosensitivity) was greater in small intestine sections compared with colon and rectum. None of slopes of the intestines studied were altered by SSE treatment at either the 2 or 5 mg/kg dose level (Fig 3).

6. Thus, SSE significantly enhances the effect of radiation on well-established hormone-independent prostate tumors, and does not sensitize the intestinal epithelial cells to radiation. This is important since intestinal epithelium is clinically relevant to the treatment of prostate cancer with irradiation. If SSE radiosensitized normal intestinal

epithelial cells, the gain in therapeutic index from using this therapeutic approach would have been jeopardized.

7. The growth of HI-LAPC-4, but not PC-3 xenograft tumor, was inhibited by SSE treatment alone, possibly due to the lack of wild type androgen receptor (AR) in PC-3 cells. We have previously found that SSE inhibits AR expression and function in LAPC-4 cells, whereas the wild type AR expression is maintained in HI-LAPC-4 cells. Given the importance of AR in prostate cancer cell growth, the inhibitory effect of SSE alone might be expected to be limited in PC-3 cell lines. However, the observation that SSE enhanced the response of both tumor types to radiation suggests that the radiosensitizing effect of SSE is independent of AR status. Since, the radiosensitizing effect of SSE is independent of AR status, SSE is likely to radiosensitize other types of tumors, in addition to prostate cancer.

8. The significant beneficial effect of the combination of the salt of an inorganic selenium-containing compound and radiation therapy on inhibition of tumor growth was observed in models of both hormone-dependent and hormone-independent prostate cancer. Therefore, it is reasonable to extrapolate the results obtained from prostate cancer mouse models to other types of cancer and to conclude that the combination of a salt of an inorganic selenium-containing compound and radiation therapy may be used to treat neoplastic disease, and to enhance sensitivity of a tumor independent of the type of tumor or the particular salt of inorganic selenium (that is, a salt of selenite or selenate).

9. Example 10 of the above referenced application states:

> Mice with well-established LAPC-4 tumors were treated with selenite alone, local tumor radiation alone, or selenite with localized tumor radiation. selenite significantly enhanced local radiation-induced tumor growth delay. The effect of the combined treatment was significantly greater than that of radiation or selenite alone. Furthermore, selenite treatment was very well tolerated, and there was no significant weight loss in the selenite

treated mice compared to the group of mice treated with local tumor irradiation alone.

10. The results of the experiment described in Example 10 refer to data obtained from a pilot experiment that was performed prior to filing of the application that first contained Example 10. This observation has been further confirmed by later work, such as that described in this document.

11. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Respectfully submitted,

Date: 10/6/09

Susan J. Knox

Susan J. Knox, M.D., Ph.D.

Enclosures: Figs. 1 - 3

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